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SEQUENCE LISTING

TECH CENTER 1600/2900

<110> TOYO BOSEKI KABUSHIKI KAISHA

<120> MODIFIED THERMOSTABLE DNA POLYMERASE

<130> 000053

<140>

<141>

<150> 2000-138796 .

<151> 2000-05-11

<160> 34

<170> PatentIn Ver. 2.1

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<211> 5342

<212> DNA

<213> Pyrococcus kodakaraensis

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<222> (156).. (5165)

<223> 1374-2453 intron, 2709-4316 intron

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cctggattgt tctacaagat tatgggggat gaaag atg atc ctc gac act gac 1

Met Ile Leu Asp Thr Asp 1 5

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Asn	Gly	Glu	Phe	Lys	Ile	Glu	Tyr	Asp	Arg	Thr	Phe	Glu	Pro	Tyr	Phe	
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acc	gcc	gag	agg	cac	ggg	acg	gtt	gta	acg	gtt	aag	cgg	gtt	gaa	aag	365
Thr	Ala	Glu	Arg	His	Gly	Thr	Val	Val	Thr	Val	Lys	Arg	Val	Glu	Lys	
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gtt	cag	aag	aag	t t c	ctc	ggg	aga	cca	gtt	gag	gtc	t gg	aaa	ctc	tac	413
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		Met														
				315		•			320			•	•	325		
									020				1.	020		
c t t	000	a t cr	ana	a a a	000	0 f f	tat	0.00	* * •	o t o	~~~		+		4	1101
		atg														1181
Leu	Pro	Met	Glu	Ala	GIn	Leu	Ser	Arg	Leu	He	Gly	Gln	Ser	Leu	Trp	
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		Ala														
J	360		- • -			365					370	-,,				
	000					000					010					
		•														
															aaa .	1325
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Cys	His	Pro	Ala	Asp	Thr	Lys	Val	Val	Val	Lys	Gly	Lys	Gly	Ile	Île	
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														-50		
tct	ttc	ctt	act	ลลล	ลลล	gtt	ลลฮ	ggr	ลลฮ	ata	ata	acc	act	_ር ር ር	ctt	1661
Ser																1001

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					Leu				•							
	·		730					735					740	- , -	- , -	
			, 00					100					, 10			
അന	aac	220	atc	tat	a a c	110	a c t	c t t	an n	aan.	n o f	000	t n a	too		9490
		-			gac											2429
GIU	GIY		vai	lyr	Asp	Leu		Leu	GIU	GIY	ınr		lyr	ıyr	Pne	
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Gly	Phe	Ile	Pro	Ser	Leu	Leu	Gly	Asp	Leu	Leu	Glu	Glu	Arg	Gln	Lys	
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a t a	aag	aag	aag	atg	aag	gcc	acg	att	gac	ccg	atc	gag	agg	aag	ctc	2669
Ile	Lys	Lys	Lys	Me t	Lys	Ala	Thr	Ile	Asp	Pro	Ile	Glu	Arg	Lys	Leu	
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														•		
ссс	gag	gaa	tgg	ctt	cca	gtc	ctc	gag	gaa	ggg	gag	gtt	cac	ttc	gtc	2765
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					Ile											
0		0.,		875		,	6		880	o.u	010		711 u	885	D y S	
				J10						•				000		
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gta									1	gag Cl	ye!	agı	RRR		gaā	2861

gtc	ccg	tcc	ttt	aac	agg	aga	act	aac	aag	gcc	gag	ctc	aag	aga	gta	2909
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Lys	Ala	Leu	He	Arg	His	Asp	Tyr	Ser	Gly.	Lys	Val	Tyr	Thr	Ile	Arg	
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Pro	Gly	Asp		Val	Ala	Vai	Pro		Arg	Leu	Glu	Leu		Glu	Arg	
			970					975					980			
000	000	a ta	a t a		a t a	~44	~~~			. 4 4						
	cac His															3149
лзіі	1113	985	LCU	USII	LCU	vai	990	rcn	LCU	ren	GIY	995	riv	GIU	UIU	
		200					J J U					330				

gaa	act	ttg	gac	atc	gtc	atg	acg	atc	cca	gtc	aag	ggt	aag	aag	aac	3197
Glu	Thr	Leu	Asp	Ile	Val	Met	Thr	Ile	Pro	Val	Lys	Gly	Lys	Lys	Asn	
1	000				1	005				1	01.0					
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ttc	ttt	aaa	ggg	atg	ctc	agg	act	ttg	cgc	tgg	a t t	ttc	gga	gag	gaa	3245
Phe	Phe	Lys	Gly	Met	Leu	Arg	Thr	Leu	Arg	Trp	Ile	Phe	Gly	Glu	Glu	
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aag	agg	ссс	aga	acc	gcg	aga	ċgc	tat	ctc	agg	cac	ctt	gag	gat	ctg	3293
Lys	Arg	Pro	Arg	Thr	Ala	Arg	Arg	Tyr	Leu	Arg	His	Leu	Glu	Asp	Leu	•
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Ser	Leu	Lys	Asn	Tyr	Arg	Arg	Leu	Tyr	Glu	Ala	Leu	Val	Glu	Asn	Val	
		1065					1070					1075				
aga	tac	aac	ggc	aac	aag	agg	gag	tac	ctc	gtt	gaa	ttc	aat	tcc	atc	3437
Arg	Tyr	Asn	Gly	Asn	Lys	Arg	Glu	Tyr	Leu	Val	Glu	Phe	Asn	Ser	Ile	
	1080					1085					1090					

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Arg	Asp	Ala	Val	Gly	Ile	Met	Pro	Leu	Lys	Glu	Leu	Lys	Glu	Trp	Lys	
1095	•			1	1100				1	1105				1	1110	
atc	ggc	acg	ctg	aac	ggc	ttc	aga	atg	aga	aag	ctc	a t t	gaa	gtg	gac	3533
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Arg	Lys	Gln	Arg	Asn	Pro	Lys	Asn	Gly	Trp	Ser	Tyr	Ser	Val	Lys	Leu	
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Tyr	Asn	Glu	Asp	Pro	Glu	Val	Leu	Asp	Asp	Met	Glu	Arg	Leu	Ala	Ser	
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Glu	Asn	Lys	Arg	Ile	Pro	Glu	Phe	Val	Phe	Thr	Ser	Pro	Lys	Gly	Val		
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cgg	ctg	gcc	ttc	ctt	gag	ggg	tac	tca	tcg	gcg	atg	gcg	acg	tcc	acc	3869	
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gaa	caa	gag	act	cag	gct	ctc	aac	gaa	aag	cga	gct	t t a	gcg	aac	cag	3917	
Glu	Gln	Glu	Thr	Gln	Ala	Leu	Asn	Glu	Lys	Arg	Ala	Leu	Ala	Asn	Gln		
1	1240				1	245				1	250						
ctc	gtc	ctc	ctc	ttg	aac	tcg	gtg	ggg	gtc	tct	gct	gta	aaa	ctt	ggg	3965	
Leu	Va l	Leu	Leu	Leu	Asn	Ser	Val	Gly	Val	Ser	Ala	Val	Lys	Leu	Gly		
125	5.				1260					1265				j	1270		
cac	gac	agc	ggc	gtt	tac	agg	gtc	tat	ata	aac	gag	gag	ctc	ccg	ttc	4013	
His	Asp	Ser	Gly	Val	Tyr	Arg	Val	Tyr	Ile	Asn	Glu	Glu	Leu	Pro	Phe		
				1275					1280					1285			
															٠		
gta	aag	ctg	gac	ลลฐ	aaa	ลลซ	aac	gcc	tac	tac	tca	cac	gtg	atc	ccc .	4061	

Val Lys Leu Asp Lys Lys Asn Ala Tyr Tyr Ser His Val Ile Pro

														-		
aag	gaa	gtc	ctg	agc	gag	gtc	ttt	ggg	aag	gtt	ttc	cag	aaa	aac	gtc	4109
Lys	Glu	Val	Leu	Ser	Glu	Val	Phe	Gly	Lys	Val	Phe	Gln	Lys	Asn	Val	
	1	305				1	310				1	1315				
agt	c c t	cag	acc	ttc	agg	aag	atg	gtc	gag	gac	gga	aga	ctc	gat	ссс	4157
Ser	Pro	Gln	Thr	Phe	Arg	Lys	Met	Val	Glu	Asp	Gly	Arg	Leu	Asp	Pro	
1	320				1	325				1	1330					
gaa	aag	gcc	cag	agg	ctc	tcc	t gg	ctc	a t t	gag	ggg	gac	gta	gtg	ctc	4205
Glu	Lys	Ala	Gln	Arg	Leu	Ser	Trp	Leu	Ile	Glu	Gly	Asp	Val	Val	Leu	
133	5			1	1340				1	1345				•	1350	
							•									
gac	cgc	gtt	gag	tcc	gtt	gat	gtg	gaa	gac	tac	gat	ggt	tat	gtc	tat	4253
Asp	Arg	Val	Glu	Ser	Val	Asp	Val	Glu	Asp	Tyr	Asp	Gly	Tyr	Val	Tyr	
			1	1355				1	360				1	1365		
gac	ctg	agc	gţc	gag	gac	aac	gag	aac	ttc	ctc	gtt	ggc	ttt	ggg	ttg	4301
Asp	Leu	Ser	Val	Glu	Asp	Asn	Glu	Asn	Phe	Leu	Val	Gly	Phe	Gly	Leu	
		1	1370				1	375				1	380			
																•
gtc	tat	gct	cac	aac	agc	tac	tac	ggt	tac	tac	ggc	tat	gca	agg	gcg	4349
Val	Tyr	Ala	His	Asn	Ser	Tyr	Tyr	Gly	Tyr	Tyr	Gly	Tyr	Ala	Arg	Ala	

cgc tgg tac tgc aag	gag tgt gca g	gag agc gta acg go	c tgg gga agg 4397
Arg Trp Tyr Cys Lys	Glu Cys Ala (Glu Ser Val Thr Al	a Trp Gly Arg
1400	1405	1410	
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gag tac ata acg atg	acc atc aag g	gag ata gag gaa aa	g tac ggc ttt 4445
Glu Tyr Ile Thr Met	Thr Ile Lys (Glu Ile Glu Glu Ly	s Tyr Gly Phe
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Lys Val Ile Tyr Ser	Asp Thr Asp (Gly Phe Phe Ala Th	r Ile Pro Gly
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		•	
gcc gat gct gaa acc	gtc aaa aag a	aag gct atg gag tt	c ctc aag tat 4541
Ala Asp Ala Glu Thr	Val Lys Lys I	Lys Ala Met Glu Ph	e Leu Lys Tyr
1450	. 14	455	1460
atc aac gcc aaa ctt	ccg ggc gcg	ctt gag ctc gag ta	c gag ggc ttc 4589
Ile Asn Ala Lys Leu	Pro Gly Ala I	Leu Glu Leu Glu Ty	r Glu Gly Phe
1465	1470	147	5
tac aaa cgc ggc ttc	ttc gtc acg	aag aag aag tat go	g gtg ata gac 4637
Tyr Lys Arg Gly Phe	Phe Val Thr I	Lys Lys Lys Tyr Al	a Val Ile Asp
1480	1485	1490	

ga	g	gaa	ggc	aag	aıa	aca	acg	cgc	gga	CII	gag	att	gıg	agg	cgi	gac	408
Gl	u	Glu	Gly	Lys	Ile	Thr	Thr	Arg	Gly	Leu	Glu	Ile	Val	Arg	Arg	Asp	
14	95					1500]	1505					1510	
t g	g	agc	gag	ata	gcg	aaa	gag	acg	cag	gcg	agg	gtt	ctt	gaa	gct	ttg	4733
Tr	р	Ser	Glu	Ile	Ala	Lys	GÌu	Thr	Gln	Ala	Arg	Val	Leu	Glu	Ala	Leu	
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				*													
c t	a	aag	gac	ggt	gac	gţc	gag	aag	gcc	gtg	agg	ata	gtc	aaa	gaa	gtt	4781
Le	u	Lys	Asp	Gly	Asp	Val	Glu	Lys	Ala	Val	Arg	Ile	Val	Lys	Glu	Val	
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Th	r	Glu	Lys	Leu	Ser	Lys	Tyr	Glu	Val	Pro	Pro	Glu	Lys	Leu	Val	Ile	
]	1545		-		1	1550]	1555	-			
сa	c	gag	cag	ata	acg	agg	gat	tta	aag	gac	tac	aag	gca	acc	ggt	ccc	4877
Ηi	S	Glu	Gln	Ile	Thr	Arg	Asp	Leu	Lys	Asp	Tyr	Lys	Ala	Thr	Gly	Pro	
	1	560				1	565				1	1570	•				
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сa	.c	gtt	gcc	gtt	gcc	aag	agg	ttg	gcc	gcg	aga	gga	gtc	aaa	a t a	cgc	4925
Ηi	s	Val	Ala	Val	Ala	Lys	Arg	Leu	Ala	Ala	Arg	Gly	Val	Lys	Ile	Arg	
15	75	ı				1580				1	1585				1	590	
сс	t	gga	acg	gtg	ata	agc	tac	atc	gtg	ctc	aag	ggc	tct	ggg	agg	a t a	4973

Pro	GIY	ınr	vaı	116	Ser	lyr	He	vai	Leu	Lys	Gly	Ser	Gly	Arg	He	
		·	1	595				1	600					1605		
ggc	gac	agg	gcg	ata	ccg	ttc	gac	gag	ttc	gac	ccg	acg	aag	cac	aag	5021
			•											His		
,	110 F									· · · · ·			-		2,0	
		1	1610	ė				1615				ļ	1620			
					-											
tac	gac	gcc	gag	tac	tac	att	gag	aac	cag	gtt	ctc	cca	gcc	gtt	gag	5069
Tyr	Asp	Ala	Glu	Tyr	Tyr	Ile	Glu	Asn	Gln	Val	Leu	Pro	Ala	Val	Glu	
	1	1625				1	630]	1635				
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Arg	Ile	Leu	Arg	Ala	Phe	Gly	Tyr	Arg	Lys	Glu	Asp	Leu	Arg	Tyr	Gln	
	1640					1645					1650					
	1010	·			,	1010				,	1000					
aag	acg	aga	cag	gtt	ggt	ttg	agt	gc t	tgg	ctg	aag	ccg	aag	gga	ac t	5165
Lys	Thr	Arg	Gln	Val	Gly	Leu	Ser	Ala	Trp	Leu	Lys	Pro	Lys	Gly	Thr	
165	5				1660]	1665				1	1670	
tgad	ectt	tcc :	attta	zttt	te e	agogi	gatas	а сс	ettta	aact	tcc	ettte	caa	aaact	tccctt	5225
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tag	ggaa	aga	ccats	gaag	at a	gaaa	tccg	g cg	gcgc	ccgg	tta	aata	cgc	t agga	atagaa	5285
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Arg Asp Lys Ile Arg Glu His Pro Ala Val Ile Asp Ile Tyr Glu Tyr
100 105 110

Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Val Pro
115 120 125

Met Glu Gly Asp Glu Glu Leu Lys Met Leu Ala Phe Asp Ile Glu Thr

130 135 140

Leu Tyr His Glu Gly Glu Glu Phe Ala Glu Gly Pro Ile Leu Met Ile 145 150 155 160

Ser Tyr Ala Asp Glu Glu Gly Ala Arg Val Ile Thr Trp Lys Asn Val

165 170 175

Asp Leu Pro Tyr Val Asp Val Val Ser Thr Glu Arg Glu Met Ile Lys
180 185 190

Arg Phe Leu Arg Val Val Lys Glu Lys Asp Pro Asp Val Leu Ile Thr
195 200 205

Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Cys Glu 210 215 220

Lys Leu Gly Ile Asn Phe Ala Leu Gly Arg Asp Gly Ser Glu Pro Lys

225	230	235	240
Ile Gln Arg Met	Gly Asp Arg Pho	e Ala Val Glu Val Lys 250	Gly Arg Ile 255
His Phe Asp Leu 260	Tyr Pro Val II	e Arg Arg Thr Ile Asn 265	Leu Pro Thr 270
Tyr Thr Leu Glu 275	Ala Val Tyr Gl	u Ala Val Phe Gly Gln 0 285	Pro Lys Glu
Lys Val Tyr Ala 290	Glu Glu Ile Th 295	r Thr Ala Trp Glu Thr 300	Gly Glu Asn
Leu Glu Arg Val	Ala Arg Tyr Se	r Met Glu Asp Ala Lys 315	Val Thr Tyr 320
Glu Leu Gly Lys	Glu Phe Leu Pr	o Met Glu Ala Gln Leu 330	Ser Arg Leu 335
Ile Gly Gln Ser 340		l Ser Arg Ser Ser Thr 345	Gly Asn Leu 350
Val Glu Trp Phe	Leu Leu Arg Ly	s Ala Tyr Glu Arg Asn 0 365	Glu Leu Ala

Pro Asn Lys Pro Asp Glu Lys Glu Leu Ala Arg Arg Gln Ser Tyr 370 375 380

Glu Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Glu Asn Ile 385 390 395 400

Val Tyr Leu Asp Phe Arg Ser Leu Tyr Pro Ser IIe IIe IIe Thr His
405 410 415

Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp
420
430

Val Ala Pro Gln Val Gly His Arg Phe Cys Lys Asp Phe Pro Gly Phe
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Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys
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Lys Lys Met Lys Ala Thr Ile Asp Pro Ile Glu Arg Lys Leu Leu Asp 465 470 475 480

Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly Tyr
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Tyr Gly Tyr Ala Arg Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser 500 505 510

Val Thr Ala Trp Gly Arg Glu Tyr Ile Thr Met Thr Ile Lys Glu Ile
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Glu Glu Lys Tyr Gly Phe Lys Val Ile Tyr Ser Asp Thr Asp Gly Phe
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Phe Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Ala
545 550 555 560

Met Glu Phe Leu Lys Tyr Ile Asn Ala Lys Leu Pro Gly Ala Leu Glu
565 570 575

Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys Lys
580 585 590

Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu
595 600 605

Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala 610 615 620

Arg Val Leu Glu Ala Leu Leu Lys Asp Gly Asp Val Glu Lys Ala Val

630

635

640

Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro
645 650 655

Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Lys Asp
660 665 670

Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala 675 680 685

Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu 690 695 700

Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Phe Asp Glu Phe 705 710 715 720

Asp Pro Thr Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln
725 730 735

Val Leu Pro Ala Val Glu Arg Ile Leu Arg Ala Phe Gly Tyr Arg Lys
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Glu Asp Leu Arg Tyr Gln Lys Thr Arg Gln Val Gly Leu Ser Ala Trp
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